



## **E.Z.N.A.® SP Plant DNA Midi Kit**

D5528-00	2 preps
D5528-01	10 preps

**May 2013**

*For research use only. Not intended for diagnostic testing.*

# **E.Z.N.A.® SP Plant DNA Midi Kit**

## **Table of Contents**

Introduction and Overview.....	2
Kit Contents/Storage and Stability.....	3
Preparing Reagents.....	4
Recommended Settings (or equivalent info).....	5
Protocol for Dried Samples.....	6
Protocol for Fresh/Frozen Samples.....	6
Troubleshooting Guide.....	14

**Manual Revision: May 2013**



# Introduction and Overview

The E.Z.N.A.® SP Plant DNA Midi Kit is specially designed for rapid and reliable isolation of high-quality total cellular DNA from plant species containing high levels of phenolic compounds and polysaccharides. Up to 500 mg wet tissue (or 125 mg dry tissue) can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of the HiBind® matrix with the speed and versatility of spin columns to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from plant tissue lysates. Purified DNA is suitable for PCR, restriction digestion, and hybridization applications. There are no organic extractions, thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

If using the E.Z.N.A.® SP Plant DNA Midi Kit for the first time, please read this booklet to become familiar with the procedures. Dry or fresh plant tissue is disrupted and lysed in a specially formulated buffer containing detergent. Binding conditions are adjusted and the sample is transferred to a HiBind® DNA Midi Column. Three rapid wash steps remove trace contaminants such as residual polysaccharides and pure DNA is eluted in water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

## Protocol Selection:

- Choose the most appropriate protocol to follow. Procedures are described for each of dried and fresh (or frozen) specimens.

<b>Dry Specimens</b> (Page 9)	Ideal for processing 125 mg powdered tissue samples. DNA yields will vary and depend on genome size, ploidy, and sample age.
<b>Fresh/Frozen Specimens</b> (Page 13)	Ideal for processing ≤500 mg fresh or frozen tissue.

## Binding Capacity:

Each HiBind® DNA Midi Column can bind up to 0.5 mg of genomic DNA. Use More than 500 mg Fresh plant samples is not recommend.

## New in this Edition:

- This manual has been edited for content and redesigned to enhance user readability.
- Equilibration Buffer (used in the Troubleshooting section) is no longer included with this kit.
- Equilibration Buffer can be replaced with 3M NaOH provided by the user.

## Kit Contents

Product	D5528-00	D5528-01
Purifications	2	10
HiBind® DNA Midi Columns	2	10
Homogenizer Midi Columns	2	10
15 mL Collection Tubes	4	20
SP1 Buffer	8 mL	40 mL
SP2 Buffer	3 mL	15 mL
SP3 Buffer	4 mL	20 mL
SPW Wash Buffer	5 mL	20 mL
RNase A	32 µL	160 µL
Elution Buffer	3 mL	12 mL
User Manual	✓	✓

## Storage and Stability

All of the E.Z.N.A.® SP Plant DNA Midi Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Store RNase A at 2-8°C. All remaining components should be stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in SP1 Buffer and SP3 Buffer. Dissolve such deposits by warming the solution at 37°C and gently shaking.

## Preparing Reagents

---

- Dilute SPW Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D5528-00	20 mL
D5528-01	80 mL

- Dilute SP3 Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D5528-00	8 mL
D5528-01	40 mL

# E.Z.N.A.® SP Plant DNA Midi Kit Protocols

## E.Z.N.A.® SP Plant DNA Midi Kit - Protocol for Dried Samples

### Materials and Equipment to be Supplied by User:

- Centrifuge capable of at least 3,000 x g
- Nuclease-free 15 mL and 50 mL high-speed centrifuge tubes
- Incubator, heat block, or water bath capable of 65°C
- 100% ethanol
- Ice bucket or cryorack for centrifuge tubes
- Optional: Liquid nitrogen for freezing/disrupting fresh samples

### Before Starting:

- Prepare SPW Wash Buffer and SP3 Buffer according to the Preparing Reagents section on Page 4
- Set an incubator, heat block, or water bath to 65°C
- Heat Elution Buffer to 65°C
- Prepare an ice bucket or cryorack

Drying allows storage of field specimens for prolonged period of time prior to processing. Samples can be dried overnight in a 45°C oven, powdered, and stored dry at room temperature. To prepare dried samples place up to 125 mg dried tissue into a 15 mL centrifuge tube (not supplied) and grind using a pellet pestle. For critical work such as PCR and cloning, pestles are best used a single time then soaked in a dilute bleach solution immediately after use until cleaning. Disposable pestles may be autoclaved several times. For standard Southern analysis, the same pestle can be reused several times to grind multiple tissue samples by rinsing with ethanol and wiping the surface clean between samples. A fine powder will ensure optimal DNA extraction and yield.

1. Transfer 30-125 mg powdered dry tissue to a 15 mL centrifuge tube.
2. Add 3.5 mL SP1 Buffer and 14 µL RNase A. Vortex at maximum speed for 20 seconds to mix thoroughly.

**Note:** Make sure to disperse all clumps by pipetting or vortexing. Clumped tissue will not be lysed properly and will result in lower DNA yields.

3. Incubate at 65°C for 30-60 minutes. Invert the tube several times during incubation.

## E.Z.N.A.® SP Plant DNA Midi Kit Protocols

---

4. Add 1.25 mL SP2 Buffer. Vortex to mix thoroughly.
5. Let sit on ice for 10 minutes.
6. Centrifuge at 3,000-5,000 x *g* for 10 minutes at room temperature.
7. Carefully transfer the supernatant to a Homogenizer Midi Column. Do not disturb the pellet.
8. Centrifuge at 3,000-5,000 x *g* for 5 minutes.

**Note:** Longer centrifugation does not improve yield. The Homogenizer Midi Column will remove most remaining precipitates and cell debris, but a small amount may pass through and form a pellet in the collection tube. Be careful not to disturb this pellet in Step 9.

9. Carefully transfer the cleared lysate to a new 15 mL centrifuge tube (not provided). Do not disturb the pellet. Measure the volume of the lysate for the next step.
10. Add 1.5 volumes SP3 Buffer. Vortex immediately at maximum speed to obtain a homogenous mixture. A precipitate may form at this point; it will not interfere with DNA isolation. Passing the mixture through a needle using a syringe or by pipetting up and down 10-15 times may break up the precipitates.

**Note:** SP3 Buffer must be diluted with 100% ethanol before use. Please see the instructions in the "Preparing Reagents" section on Page 4.

### Optional Protocol for Column Equilibration:

1. Add 1 mL 3M NaOH to a HiBind® DNA Midi Column.
  2. Let sit for 4 minutes.
  3. Centrifuge at 3,000 x *g* for 3 minutes.
  4. Discard the filtrate and reuse the Collection Tube.
- 
11. Transfer 3.5 mL cleared lysate (including any precipitates) from Step 10 to the HiBind® DNA Midi Column.

## E.Z.N.A.® SP Plant DNA Midi Kit Protocols

---

12. Centrifuge at 3,000-5,000 x *g* for 5 minutes.
13. Discard the filtrate and reuse the collection tube.
14. Repeat Steps 11-13 until all of the cleared lysate has been transferred to the HiBind® DNA Midi Column.
15. Add 3 mL SPW Wash Buffer.

**Note:** SPW Wash Buffer must be diluted with 100% ethanol prior to use. Please see the instructions in the “Preparing Reagents” section on Page 4.
16. Centrifuge at 3,000-5,000 x *g* for 4 minutes.
17. Discard the filtrate and reuse the collection tube.
18. Repeat Steps 15-17 for a second SPW Wash Buffer wash step.
19. Centrifuge the empty HiBind® DNA Midi Column at 5,000 x *g* for 10 minutes to dry the column matrix.

**Note:** It is important to dry the HiBind® DNA Midi Column matrix before elution. Residual ethanol may interfere with downstream applications.
20. Transfer the HiBind® DNA Midi Column to a new nuclease-free 15 mL centrifuge tube (not provided).
21. Add 400-500 µL Elution Buffer heated to 65°C directly to the center of the column matrix.

**Note:** Smaller volumes will significantly increase DNA concentration but result in lower yields.

## E.Z.N.A.® SP Plant DNA Midi Kit Protocols

---

22. Let sit at room temperature for 5 minutes.

**Note:** To increase DNA concentration, add the buffer and incubate the column at 60-70°C for 5 minutes before elution.

23. Centrifuge at 3,000-5,000 x *g* for 3 minutes.

24. Repeat Steps 21-23 for a second elution step.

**Note:** This step may be performed using another 50 mL centrifuge tube to maintain a higher DNA concentration in the first eluate. Alternatively, DNA concentration can be increased by using the first eluate for a second elution.

25. Store DNA at -20°C.

# E.Z.N.A.® SP Plant DNA Midi Kit Protocols

---

## E.Z.N.A.® SP Plant DNA Midi Kit - Protocol for Fresh/Frozen Samples

### Materials and Equipment to be Supplied by User:

- Centrifuge capable of at least 3,000 x *g*
- Nuclease-free 15 mL and 50 mL high-speed centrifuge tubes
- Water bath capable of 65°C
- 100% ethanol
- Ice bucket or cryorack for centrifuge tubes
- Liquid nitrogen for freezing/disrupting samples

### Before Starting:

- Prepare SPW Wash Buffer and SP3 Buffer according to the Preparing Reagents section on Page 4
- Set an incubator, heat block, or water bath to 65°C
- Heat Elution Buffer to 65°C
- Prepare an ice bucket or cryorack

Note: Use extreme caution when handling liquid nitrogen.

This protocol is suitable for most fresh or frozen tissue samples allowing more efficient recovery of DNA. However, due to the tremendous variation in water and polysaccharide content of plants, sample size should be limited to 500 mg. Best results are obtained with young leaves or needles.

To prepare samples, collect tissue in a 15 mL mortar and freeze by dipping in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using a clean pestle. Alternatively, one can allow liquid nitrogen to evaporate and then store samples at -70°C for later use. For critical work such as PCR and cloning, pestles are best used a single time then soaked in a dilute bleach solution immediately after use until clean. Disposable pestles may be autoclaved several times. For standard Southern analysis, the same pestle can be reused several times to grind multiple tissue samples by rinsing with ethanol and carefully wiping the surfaces clean between samples.

1. Transfer up to 500 mg ground plant tissue to a 15 mL centrifuge tube (not supplied).

## E.Z.N.A.® SP Plant DNA Midi Kit Protocols

---

2. Add 2.5 mL SP1 Buffer and 14  $\mu$ L RNase A. Vortex at maximum speed for 20 seconds to mix thoroughly.

**Note:** Make sure to disperse all clumps by pipetting or vortexing. Clumped tissue will not be lysed properly and will result in lower DNA yields.

3. Incubate at 65°C for 10 minutes. Invert the tube several times during incubation.
4. Add 870  $\mu$ L SP2 Buffer. Vortex to mix thoroughly.
5. Let sit on ice for 10 minutes.
6. Centrifuge at 3,000-5,000  $\times g$  for 10 minutes at room temperature.
7. Carefully transfer the supernatant to a Homogenizer Midi Column. Do not disturb the pellet.
8. Centrifuge at 3,000-5,000  $\times g$  for 5 minutes.

**Note:** Longer centrifugation does not improve yield. The Homogenizer Midi Column will remove most remaining precipitates and cell debris, but a small amount may pass through and form a pellet in the collection tube. Be careful not to disturb this pellet in Step 9.

9. Carefully transfer the cleared lysate to a new 15 mL centrifuge tube (not provided). Do not disturb the pellet. Measure the volume of the lysate for the next step.
10. Add 1.5 volumes SP3 Buffer. Vortex immediately at maximum speed to obtain a homogenous mixture. A precipitate may form at this point; it will not interfere with DNA isolation. Passing the mixture through a needle using a syringe or by pipetting up and down 10-15 times may break up the precipitates.

**Note:** SP3 Buffer must be diluted with 100% ethanol before use. Please see the instructions in the "Preparing Reagents" section on Page 4.

# E.Z.N.A.® SP Plant DNA Midi Kit Protocols

---

## Optional Protocol for Column Equilibration:

1. Add 1 mL 3M NaOH to a HiBind® DNA Midi Column.
  2. Let sit for 4 minutes.
  3. Centrifuge at 3,000 x *g* for 3 minutes.
  4. Discard the filtrate and reuse the Collection Tube.
- 
11. Transfer 3.5 mL cleared lysate (including any precipitates) from Step 10 to the HiBind® DNA Midi Column.
  12. Centrifuge at 3,000-5,000 x *g* for 5 minutes.
  13. Discard the filtrate and reuse the collection tube.
  14. Repeat Steps 11-13 until all of the cleared lysate has been transferred to the HiBind® DNA Midi Column.
  15. Add 3 mL SPW Wash Buffer.
- Note:** SPW Wash Buffer must be diluted with 100% ethanol prior to use. Please see the instructions in the “Preparing Reagents” section on Page 4.
16. Centrifuge at 3,000-5,000 x *g* for 4 minutes.
  17. Discard the filtrate and reuse the collection tube.
  18. Repeat Steps 15-17 for a second SPW Wash Buffer wash step.
  19. Centrifuge the empty HiBind® DNA Midi Column at 5,000 x *g* for 10 minutes to dry the column matrix.

**Note:** It is important to dry the HiBind® DNA Midi Column matrix before elution. Residual ethanol may interfere with downstream applications.

## E.Z.N.A.® SP Plant DNA Midi Kit Protocols

---

20. Transfer the HiBind® DNA Midi Column to a new nuclease-free 15 mL centrifuge tube (not provided).

21. Add 400-500 µL Elution Buffer heated to 65°C directly to the center of the column matrix.

**Note:** Smaller volumes will significantly increase DNA concentration but result in lower yields.

22. Let sit at room temperature for 5 minutes.

**Note:** To increase DNA concentration, add the buffer and incubate the column at 60-70°C for 5 minutes before elution.

23. Centrifuge at 3,000-5,000 x *g* for 3 minutes.

24. Repeat Steps 21-23 for a second elution step.

**Note:** This step may be performed using another 50 mL centrifuge tube to maintain a higher DNA concentration in the first eluate. Alternatively, DNA concentration can be increased by using the first eluate for a second elution.

25. Store DNA at -20°C.

# Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Clogged column	Debris carryover	Following precipitation with SP2 Buffer, make sure no particulate material is transferred.
	Sample too viscous	Do not exceed suggested amount of starting material. Alternatively, increase the amount of SP1 Buffer and SP2 Buffer.
	Incomplete precipitation following addition of SP2 Buffer	Increase RCF or centrifugation time after the addition of SP2 Buffer.
Problem		Solution
Low DNA yield	Incomplete disruption of starting material	For both dry and fresh samples, obtain a fine homogeneous powder before adding SP1 Buffer.
	Poor lysis of tissue	Decrease amount of starting material or increase amount of SP1 Buffer and SP2 Buffer.
	DNA remains bound to column	Increase elution volume and incubate column at 65°C for 5 minutes before centrifugation.
	DNA washed off	Dilute SPW Wash Buffer by adding appropriate volume of 100% ethanol prior to use (Page 4).
Problem		Solution
Problems in downstream applications	Salt carryover	SPW Wash Buffer must be at room temperature.
	Ethanol carryover	Following the second SPW Wash Buffer step, ensure that the column is dried by centrifuging 10 minutes at maximum speed.

**Notes:**

---